Comparison of the Efficiency of Biological Transmission of *Anaplasma marginale* (Rickettsiales: Anaplasmataceae) by *Dermacentor andersoni* Stiles (Acari: Ixodidae) with Mechanical Transmission by the Horse Fly, *Tabanus fuscicostatus* Hine (Diptera: Muscidae)

GLEN A. SCOLES,[1] J. ALLEN MILLER,[2] AND LANE D. FOIL[3]

J. Med. Entomol. 45(1): 109–114 (2008)

ABSTRACT Mechanical transmission of *Anaplasma marginale* by horse flies (Tabanidae) is thought to be epidemiologically significant in some areas of the United States. We compared the relative efficiencies of mechanical transmission of *Anaplasma marginale* by the horse fly, *Tabanus fuscicostatus* Hine, during acute infection ($\approx 10^7$ to $\approx 10^9$ infected erythrocytes [IE]/ml blood) with biological transmission by *Dermacentor andersoni* Stiles in the persistent phase of infection ($\approx 10^{2.5}$ to $\approx 10^6$ IE/ml). Transmission of *A. marginale* was not observed when horse flies were partially fed on an acutely infected donor calf and immediately transferred to susceptible calves to complete their blood meal. Ticks that were acquisition fed on the same donor host after it reached the persistent phase of infection successfully transmitted *A. marginale* when transferred to the same recipient calves that failed to acquire infection after fly feeding. Failure of fly-borne mechanical transmission at a rickettsemia >240-fold higher than that from which ticks transmitted with 100% efficiency shows that tick-borne biological transmission is at least two orders of magnitude more efficient than mechanical transmission by horse flies.

KEY WORDS mechanical transmission, ticks, horse flies, anaplasmosis

Anaplasma marginale Theiler is a biologically transmitted, tick-borne rickettsial pathogen of cattle. Fly-borne mechanical transmission has also been shown, and the epidemiology of disease caused by *A. marginale* in the southeastern United States suggests that mechanical transmission plays an important role in the spread of the pathogen during outbreaks (Howell et al. 1941, Wilson 1968, Morley and Hugh-Jones 1989). Biting flies in several different genera have been shown experimentally to be mechanical vectors (Ewing 1981, Potgieter et al. 1981, Hawkins et al. 1982). In a recent study to quantify the relative efficiency of tick-borne biological transmission versus biting fly-borne mechanical transmission, stable flies (*Stomoxys calcitrans*) failed to transmit *A. marginale* at rickettsemias >300-fold higher than the level at which tick-borne transmission was 100% efficient (Scoles et al. 2005b). Horse flies (tabanids) have long been considered to be the most important mechanical vectors of *A. marginale* because their relatively large mouthparts and their mode of feeding provide more opportunity for mechanical transmission than other biting flies. Several different species of tabanids have been incriminated as vectors of *A. marginale* (Ewing 1981, Potgieter et al. 1981, Hawkins et al. 1982, Foil and Gorham 2000); however, studies to quantify the efficiency of horse fly-borne mechanical transmission have not been done.

Cattle infected with *A. marginale* first develop an acute infection that may reach a peak of 10^9 infected erythrocytes (IE) per milliliter of blood. Clinical disease occurs during acute infection ($\approx 10^7$ to $\approx 10^9$ IE/ml) and can be characterized by anemia, weight loss, abortion, and in many cases, death. Cattle that survive acute infection maintain a persistent low-level rickettsemia for life. During persistent infections, the rickettsemia cycles between $\approx 10^{2.5}$ and $\approx 10^6$ IE/ml, but cattle are otherwise free of clinical disease and can serve as reservoirs of the pathogen.

Within competent tick vectors, *A. marginale* replicates both within the gut epithelium and in the salivary gland acini, culminating in levels of 10^4–10^6 organisms per salivary gland pair during subsequent transmission feeding, regardless of the level of infection of the acquisition host (Lohr et al. 2002, Futse et al. 2003,

[1] Corresponding author: USDA–ARS, Animal Disease Research Unit, Washington State University, Pullman, WA 99164 (e-mail: scoles@vetmed.wsu.edu).
[2] USDA–ARS, Knipling-Bushland U.S. Livestock Insects Research Laboratory, Kerrville, TX 78028-9184.
[3] Department of Entomology, Louisiana State University, Baton Rouge, LA 70803-1710.

Scoles et al. 2005a). For this reason, biological vectors are capable of very efficiently acquiring and transmitting the infection from persistently infected cattle. In contrast, transmission by biting flies is purely mechanical and is thus directly dependent on the level of rickettsemia in the host at the time of feeding; therefore, fly-borne mechanical transmission is presumed to be possible only during the acute phase of infection when the rickettsemia is at its highest.

Tabanids are common blood-feeding flies that have been implicated in mechanical transmission of several different pathogens. These flies have all the characteristics necessary to make them good mechanical vectors: they are highly mobile, have frequent interrupted feedings, and have large easily contaminated mouthparts for transfer of infectious material (Foil 1989). Tabanids inflict a painful bite, resulting in many interrupted feedings because of host defensive behaviors. When blood feeding is interrupted, flies may immediately contact another nearby host because cattle that are under fly feeding pressure tend to cluster closely together (Foil 1989, Davies 1990). This pattern of frequent interrupted feedings and subsequent rapid movement between hosts provides an ideal opportunity for mechanical transmission of *A. marginale* from infected to uninfected hosts.

Nine different species of Tabanidae, including *Tabanus fuscicostatus* (Hine), have been shown to mechanically transmit *A. marginale* (Ewing 1981, Hawkins et al. 1982). However, in most of the previous studies where transmission by tabanids has been shown, acutely infected splenectomized cattle were used as acquisition hosts. Splenectomy of cattle results in rickettsemias one to two orders of magnitude higher than those that would be seen in naturally infected, spleen-intact animals. Although use of splenectomized animals shows the potential for transmission, it also produces an unrealistic bias in favor of transmission.

In this study, we follow-up our previous study on mechanical transmission of *A. marginale* by stable flies with a study comparing mechanical transmission by *T. fuscicostatus* with biological transmission by *D. andersoni.* Using an experimental design similar to the previous study (Scoles et al. 2005b), we examined the potential for mechanical transmission of *A. marginale* by horse flies using quantitative polymerase chain reaction (qPCR) to estimate the amount of inoculum on the mouthparts of flies fed on an acutely infected calf. We established a minimum difference in the efficiency of biological versus mechanical transmission by comparing mechanical transmission by horse flies during the acute phase of infection with biological transmission by *D. andersoni* during the persistent phase of infection.

Materials and Methods

***Anaplasma marginale* Strains.** The St. Maries strain of *A. marginale* used in these experiments originated from *Dermacentor andersoni* ticks collected from a naturally infected bull in St. Maries, ID (Eriks et al. 1994). The St. Maries strain is a well-characterized tick transmissible strain that we have used in several previous studies. Although there are *A. marginale* strains that are not transmissible by ticks and are presumably maintained by mechanical transmission, we have no reason to suspect that a tick transmissible strain will not also be mechanically transmissible. Because of this, we chose to use the same tick transmissible strain for both tick-borne and fly-borne transmission to eliminate variation among strains as a variable between the two modes of transmission.

Horse Flies. *Tabanus fuscicostatus* were field collected in the Thistelthwaite Wildlife Management Area, St. Landry Parish, LA, 18–19 June 2004 using canopy traps baited with dry ice. After collection, flies were held in coolers in 1-gal paper cartons with sugar cubes and water-soaked cotton. Flies were transported to Kerrville and used for feeding trials within 1–3 d of collection.

Rocky Mountain Wood Ticks. Ticks used in these studies were obtained from a laboratory colony of *D. andersoni* established from ticks originally collected at the Reynolds Creek watershed in Owyhee Co., southwestern Idaho. These ticks have been maintained in colony for >15 yr (20+ generations) and are competent vectors of the St. Maries strain of *A. marginale.* Ticks were reared on rabbits and cattle at the University of Idaho Holm Research Center and shipped overnight to Kerrville Texas for use in the experiment.

Cattle. All cattle used in these experiments were cared for following procedures approved by the Knipling-Bushland U.S. Livestock Insects Research Laboratory Animal Care and Use Committee. All calves were confirmed to be free of *A. marginale* infection using a commercially available competitive enzyme-linked immunosorbent assay (cELISA; VMRD, Pullman, WA) and nested PCR assay (nPCR; see below) before use. One Hereford calf was used as an acquisition host; calf 437 was inoculated with the St. Maries strain of *A. marginale* on 2 June 2004. The transmission studies were conducted using uninfected Hereford calves 446, 447, 449, and 450 at ≈5–6 mo of age. Calf 449 died of causes unrelated to *A. marginale* infection on day 175 after fly transmission feeding (this calf was negative for *A. marginale* by PCR at the time of death) and was replaced with uninfected calf 478 for the tick feeding portion of the experiment.

Acquisition and Transmission Feeding by Horse Flies During Acute Infection. Blood was collected from the calves on the day of fly feeding, and the percent of parasitized erythrocytes (PPEs) was determined using Giemsa-stained blood smears. The number of *A. marginale* copies per milliliter of blood was determined using qPCR (see below). For feeding, flies were placed individually in 30-ml clear plastic creamer cups covered with an index card. Cups with flies were placed against the shaved side of the *A. marginale*–infected calf and the index card was withdrawn, exposing the fly to the calf. Eighteen flies were allowed to feed individually on the infected calf and were dissected immediately, without transmission, to determine the amount of *A. marginale* on the mouth-

parts in the absence of transmission feeding. For the transmission trial, flies were fed on the infected host (i.e., the acquisition feeding) until blood could be seen in the gut through the abdominal pleural membrane. Feeding was interrupted by sliding the index card between the calf and the cup. Taking care to avoid contamination of the bite site with blood from the previous host, partially fed flies were immediately moved to a transmission host and allowed to continue feeding to repletion (i.e., the transmission feeding). After feedings, flies were immediately frozen on dry ice for later dissection.

Previously published data on the amount of blood left on the mouthparts of a tabanid after feeding (Foil et al. 1987, Knaus et al. 1993), data on the average levels of infection of acutely infected spleen intact cattle (French et al. 1998), and data on the minimum infectious dose of *A. marginale* for cattle (Scoles et al. 2005b) all led to the calculation that a single fly feeding on an acutely infected calf could transmit an infectious dose. For these reasons, and because our experimental design was intended to span a threshold value for the number of flies capable of transmitting, we chose one fly as our minimum exposure for each of two calves (449 and 450) and 10 flies as a $\log_{10}$ increase in dose of flies feeding for each of two additional calves (446 and 447). Numbers of flies available for use in this experiment did not permit us to test a 2 $\log_{10}$ increase in dose of flies feeding (i.e., 100 flies).

After transmission feeding on uninfected cattle, flies were dissected and tested for *A. marginale* by PCR as described below. Guts were tested to confirm feeding on the infected host; mouthparts were tested to determine whether they retained any *Anaplasma* after the transmission feeding. Flies were held frozen at −30°C before dissection and thawed on ice a few at a time for dissection. Each fly was dissected on a fresh piece of dental wax with clean forceps (Scoles et al. 2005a) and a clean razor blade for each fly. The mouthparts, including the labela, labium, and a portion of the rostrum, were removed dry, and a drop of Hank's balanced salt solution (Sigma Aldrich, St. Louis, MO) was added and the gut floated out. Guts were rinsed in a drop of clean saline to remove surface contamination. The mouthparts and guts were placed individually in 100 μl of proteinase k buffer (0.01 M Tris, pH 7.8; 0.005 M EDTA; 0.5% SDS) with 100 μg/ml enzyme and stored frozen until DNA preps were made.

Calves were monitored for the development of *A. marginale* infection for 175 d after fly transmission feedings by weekly examination of Giemsa-stained blood smears, by nested PCR (described below) and by cELISA (VMRD).

Effect of Time After Feeding on the Amount of *A. marginale* on Mouthparts. A group of 64 flies was fed in pairs to determine the effect of a time delay after feeding on the amount of *A. marginale* on the mouthparts. The mouthparts of the first fly from each pair were dissected and frozen immediately after feeding, and the mouthparts of the second fly were removed and frozen after holding the fly in its feeding cup at barn temperature for 20 min. Flies were fed, as previously described, until blood was seen entering the gut.

Effect of Feeding Time on Amount of *A. marginale* on Mouthparts. To determine whether feeding time had an effect on the amount of *A. marginale* on the mouthparts, a group of flies was fed either to repletion ($n = 10$), to the point where blood was first seen entering the gut ($n = 10$), or were allowed to probe but not begin feeding ($n = 5$). These flies were frozen immediately after feeding and dissected, and the mouthparts were tested as previously described.

Tick Transmission. One hundred seventy-five days after fly transmission feeding failed to infect any of the four calves, tick-borne transmission was attempted using the same acquisition host (now persistently infected) and transmitting to the same transmission hosts that failed to acquire infection after fly feeding. During feeding, male ticks were confined under stockinet sleeves attached to the sides of the calves. Ticks were acquisition fed for 7 d (7–14 December 2004) on the St. Maries strain–infected calf 437, after it had entered the persistent phase of infection (188 d PI). The ticks were removed and held off the host for 24 h at 25°C, 98% RH, before being transmission fed for 7 d on three of the same four calves that failed to acquire infection when horse flies were fed on them 175 d previously, along with one additional calf. One tick each was fed on calves 478 (replacement for 449, which died of unrelated causes) and 450 (the same calf that had earlier been transmission fed on by one horse fly), and 10 ticks each were fed on calves 446 and 447 (the same calves that had earlier been transmission fed on by 10 horse flies). A sample of 25 ticks was dissected before transmission feeding; all of the ticks from each calf were dissected after the transmission feeding. The guts and salivary glands of the transmission ticks were placed separately in 100 μl of proteinase k buffer, as described above, and stored frozen until DNA preps were made. After tick transmission, feeding calves were monitored for infection with *A. marginale* as previously described.

DNA Preparation and PCR. DNA was prepared from dissected fly (mouthparts or gut) and tick (gut or salivary gland) tissues as previously described (Scoles et al. 2005b). Fly and tick tissues were tested for *A. marginale* using a nested PCR that targets the *A. marginale msp5* gene as previously described (Scoles et al. 2005a). Based on repeated PCR amplification of the serially diluted cloned *msp5* fragment described below, we determined that the sensitivity threshold for this nested PCR is <10 copies. Because 5 μl of sample was used in each PCR reaction, representing 1/10 of the sample, the lower threshold for detection per mouthpart preparation (i.e., the mouthparts of one fly) was <100 organisms (10^2).

Blood samples from the acquisition hosts, a sample of the tick salivary glands, and all of the fly mouthparts that were positive for *A. marginale* by nested PCR were tested by qPCR using the TaqMan protocol previously described (Scoles et al. 2005a). Samples were run in triplicate and expressed as the mean for the three replicates. The detection threshold for this

Table 1. Contamination of horse fly mouthparts and guts with *A. marginale* with and without transmission feeding determined by nPCR

Transmission calf	No. flies	*A. marginale* infection					
		Mouthparts			Gut		
		n	X	Percent infected	*n*	X	Percent infected
Ntf	18[a]	18	18	100	18	18	100
449	1[b]	1	1	100	1	1	100
450	1[b]	1	1	100	1	1	100
446	10[b]	9[c]	9	100	9	9	100
447	10[b]	9[c]	8	89	9	9	100

[a] Flies were acquisition fed on 437 during the acute phase of infection (4.1×10^7 IE/ml) but not transmission fed.

[b] Flies acquisition fed and immediately transferred to susceptible transmission calves to complete their blood meals.

[c] One fly lost after transmission feeding.

n, no. examined; X, no. PCR positive for *A. marginale*; ntf, not transmission fed.

qPCR assay is between 10 and 100 copies. As stated above, 5 μl (1/10 of the sample) was used in each PCR reaction; therefore, the lower threshold for quantification with this qPCR test is <1,000 organisms (10^3) per mouthpart preparation. The standard curve for this qPCR was constructed using dilutions from 10^6 to 10^3 copies; samples predicted to be $>10^6$ (i.e., most blood samples) were diluted 1:10 and 1:100 so that the results would fall within the range of the standard curve. Because they have different sensitivities, the two PCR tests are complementary to one another; nPCR can be used to detect infection when it is at levels below the threshold for quantification by qPCR.

As a control for the quality of the DNA preparation, all samples that were PCR negative for *A. marginale* were tested by PCR for the presence of fly or tick DNA as described (Scoles et al. 2005a).

Statistical Analysis. Means were compared with Student's *t*-test on log-transformed data. Log transformation is necessary to linearize the exponential data (i.e., the qPCR results) to better fit the assumption of normality for the *t*-test.

Results

Acquisition by Horse Flies Feeding During Acute Infection. *Anaplasma marginale* was detected by nested PCR on the mouthparts of all 18 flies that were fed on the acutely infected calf (437) and dissected immediately, without transmission feeding (Table 1). However only 11 of these mouthparts had levels that could be quantified by qPCR. The level of infection of the calf at the time of feeding was 4.1×10^7 organisms/ml blood. These flies had an average of $1.05 \pm 1.9 \times 10^3$ organisms on their mouthparts or the equivalent of 25.6 nl of blood (range, 7.82×10^1– 6.63×10^3 or 1.9–161.7 nl of blood).

Fly Transmission Trial. Transmission failed when flies were partially fed on the acutely infected host and transferred immediately to uninfected cattle and allowed to feed to repletion. Two cattle that were fed on by 10 flies each and two cattle that were fed on by a single fly each failed to acquire infection. All but 1 of the 20 flies tested (2 were lost after transmission feeding) had levels of *A. marginale* that were detectable by nPCR on the mouthparts after transmission feeding (Table 1). Six transmission fed flies had levels that could be quantified by qPCR, averaging $2.44 \pm 2.84 \times 10^2$ organisms per fly, or the equivalent of 5.95 nl of blood of the acquisition host remaining (range, 2.05×10^1–7.79×10^2 or 0.5–19.0 nl). Cattle were monitored for 175 d after the transmission attempt; *A. marginale* could not be detected by stained blood smear, nested PCR, or serology.

Effect of Time After Feeding on the Amount of *A. marginale* on Mouthparts. *Anaplasma marginale* was detected on the mouthparts of 28/32 (87.5%) flies fed and dissected immediately and 29/32 (90.6%) of the flies dissected after holding for 20 min. Of the flies dissected immediately, 19 had quantifiable levels of *A. marginale* on their mouthparts, with an average of $7.52 \pm 7.58 \times 10^2$ organisms per fly. Sixteen of the flies dissected after holding for 20 min had quantifiable levels of *A. marginale* on their mouthparts, with an average of $1.44 \pm 2.40 \times 10^3$ organisms per fly. Although flies that were held for 20 min appeared to have had, on average, nearly twice as much *A. marginale* on their mouthparts, there was no statistical difference between the two groups (Student's *t*-test performed on log-transformed data; $t = 0.3508$; df = 33; $P > 0.7279$).

Effect of Feeding Time on *A. marginale* on Mouthparts. Five flies were allowed to probe but were prevented from feeding; two of the five flies had nested PCR positive mouthparts, but none had quantifiable amounts of *A. marginale.* One of the 10 flies that had their feeding interrupted because blood was seen entering the gut escaped and was lost; 7 of the 9 remaining flies were nPCR positive, and 5 of these had quantifiable amounts of *A. marginale* on their mouthparts, with an average of $5.38 \pm 2.27 \times 10^2$ organisms. Nine of 10 of the flies that were allowed to feed to repletion had nested PCR-positive mouthparts, and 8 of these had quantifiable amounts averaging $1.25 \pm 1.30 \times 10^2$. Even though the flies that fed to repletion appeared to have had, on average, more organisms on their mouthparts than the flies that had their feeding interrupted, the difference between the two groups was not significant (Student's *t*-test performed on log-transformed data; $t = 0.9245$; df = 11; $P = 0.3750$). Feeding times for these flies ranged from 0 (probe only) to 222 s, but there was no correlation between length of feeding and amount of *A. marginale* on the mouthparts.

Tick-borne Transmission. *Anaplasma marginale* was successfully transmitted to all four calves by ticks. The average number of *A. marginale* per milliliter of blood over the 7-d tick acquisition feed was 1.69×10^5, >240-fold lower than the level of infection at the time fly transmission was attempted. All of the transmission-fed ticks had nPCR-positive guts and salivary glands (Table 2).

Table 2. Infection of ticks by *A. marginale* after tick transmission feeding[a]

Calf	No. ticks	Guts			Salivary glands		
		n	X	Percent	*n*	X	Percent
Prefeed[b]	25	25	19	76.0	25	14	56.0
478[c]	1	1	1	100	1	1	100
450	1	1	1	100	1	1	100
446	10	10	10	100	10	10	100
447	10	10	9	90.0	10	9	90.0

[a] Ticks were acquisition fed on 437 for 7 d during the persistent phase of infection (avgerage 1.69×10^5 *A. marginale* genome copies/ml) and transferred to calves for 7-d transmission feeding.

[b] Ticks that were dissected before transmission feeding and were not transmission fed.

[c] Replacement calf for 449, which died of causes unrelated to infection with *A. marginale.*

n, no. of ticks dissected; X, no. of ticks positive.

Discussion

The failure of horse flies to transmit *A. marginale* from an acutely infected calf followed by successful tick-borne transmission from the same calf nearly 6 mo later after it had reached a 240-fold lower rickettsemia during the persistent phase of infection shows that tick-borne biological transmission is considerably more efficient than fly-borne mechanical transmission. A single *D. andersoni* was able to efficiently transmit *A. marginale* after feeding at a rickettsemia that was two orders of magnitude lower than the level at which fly-borne mechanical transmission by either 1 or 10 flies failed.

Using an ELISA methodology, Foil et al. (1987) estimated that the amount of blood retained on the mouthparts of *T. fuscicostatus* after blood feeding was 10.0 ± 5.0 nl. Radioisotope-based methods were used to estimate that 12.5 nl of blood was retained on the mouthparts after a short interrupted feeding (Knaus et al. 1993). Using qPCR in this study, we estimated that the average volume of blood retained on the mouthparts after the initial (or "acquisition") feeding to be 25.6 nl and that the average amount of blood left from the original acquisition host after the subsequent "transmission" feeding was 5.95 nl. This suggests that ≈20 nl was transferred from the first host to the second, a very similar result to that found in the previous studies (Foil et al. 1987, Knaus et al. 1993).

By using a qPCR designed to target a single copy *A. marginale* gene, we were able to determine the number of *A. marginale* organisms per milliliter of blood. Because *A. marginale* replicates within IE after invasion, the number of organisms per IE may range as high as 16; however, it has been estimated that, on average, each IE contained 2–4 organisms (Ristic and Watrach 1963, French et al. 1998). Using this approximation as a correction factor, the number of IE per milliliter of blood at the time of fly feeding can be estimated to have been between 1×10^7 and 2×10^7.

The minimal infectious dose (MID) of *A. marginale* IEs needed for infection by intravenous (IV) challenge has been estimated to be <100 IE (cited in Scoles et al. 2005b as an unpublished observation of G. H. Palmer). Based on this, if ≈20 nl of blood is transferred from one host to the next when *T. fuscicostatus* feeds, a rickettsemia of 5×10^6 IE/ml would be needed for a single fly to transmit one MID. Individual flies in our study should have transferred one to two times the MID at the rickettsemia of the calf at the time transmission was attempted. However, 10 flies fed at a rickettsemia that was twice this threshold level failed to transmit, suggesting that the efficiency for tabanid-borne transmission is at least 10- to 20-fold lower than predicted. Reduced efficiency may be related to the portal of infection; the MID determined using IV inoculation may be lower than the MID for organisms introduced intradermally by tabanid feeding. Innate immune response mechanisms in the skin may result in a higher MID for this portal of entry.

Although an infection of 5×10^6 IE/ml is below the threshold for reliable microscopic detection, it is near the high end of the range of rickettsemias expected in persistently infected cattle. Among the older papers reviewed by Ewing (1981) that we have been able to examine, only one (Lotze 1944) attempted to show tabanid-borne mechanical transmission from persistently infected spleen intact cattle. The rickettsemia of infected cattle was monitored with stained blood smears (detection threshold 10^6–10^7 IE/ml), and transmission was attempted when the rickettsemia rose to detectable levels. Although transmission failed when the rickettesemia was below microscopic detection, transmission was shown three different times when the PPE rose to 0.09–0.19% (Lotze 1944). This is $\approx 6.3 \times 10^6$–1.3×10^7 IE/ml, only slightly higher than our estimated threshold for mechanical transmission of 5×10^6 IE/ml. In this study, transmission failed with 10 bites near this threshold, whereas in the older study, transmission was accomplished with 72, 59, and 81 bites from 17, 15, and 13 flies, respectively (i.e., each fly had multiple opportunities to bite) (Lotze 1944). Increasing the number of fly bites may increase the probability that one or more of the bites will carry a dose that is above the threshold required for infection. In some areas during certain seasons of the year, cattle can receive thousands of bites over the course of the season, and between 2.0 and 12.3% of tabanids (depending on the species) transfer from one host to another during feeding (Foil 1983, Barros and Foil 2007).

Our data on the effect of time after feeding on the amount of *A. marginale* on mouthparts suggests that there is no reduction in the number of organisms on the mouthparts of the flies for at least 20 min after flies have fed on an infected host. Although viability studies of *A. marginale* on fly mouthparts would be needed for corroboration, these data suggest that transfer between hosts may not need to be immediate for transmission to occur.

This study showed that the efficiency of mechanical transmission by direct transfer of flies from an infected to a susceptible host is at least 10- to 20-fold lower than would be predicted by the infection level of the host and the blood volume transferred. Furthermore, we showed that tabanid-borne mechanical transmission is

at minimum two orders of magnitude less efficient than tick-borne biological transmission. Because horse fly–borne mechanical transmission of *A. marginale* is generally believed to be epidemiologically important in some areas, additional studies will be necessary to clarify the conditions under which mechanical transmission occurs and the role that it plays in the epidemiology of *A. marginale.*

Acknowledgments

R. Horn and S. Davis (ADRU) and C. Younger (LSU) provided superior technical assistance. We thank J. E. George at the USDA–ARS, Knipling-Bushland U.S. Livestock Insects Research Laboratory, Kerrville, TX, for providing facilities and cattle for the experiment; J. M. Pound, also of the Kerrville Laboratory, for assistance with cattle bleeding; and D. P. Knowles, G. H. Palmer, and two anonymous reviewers for reviewing earlier drafts of the manuscript. This work was supported by USDA–ARS CRIS 5348-32000-016-00D.

References Cited

Barros, A. T., and L. D. Foil. 2007. The influence of distance on movement of tabanids (Diptera: Tabanidae) between horses. Vet. Parasitol. 144: 380–384.

Davies, C. R. 1990. Interrupted feeding of blood-sucking insects: causes and effects. Parasitol. Today 6: 19–22.

Eriks, I. S., D. Stiller, W. L. Goff, M. Panton, S. M. Parish, T. F. McElwain, and G. H. Palmer. 1994. Molecular and biological characterization of a newly isolated *Anaplasma marginale* strain. J. Vet. Diagn. Invest. 6: 435–441.

Ewing, S. A. 1981. Transmission of *Anaplasma marginale* by arthropods, pp. 425–434. *In* R. J. Hidalgo and W. E. Jones (eds.), Proceedings of the seventh national anaplasmosis conference, Starkville, MS.

Foil, L. D. 1983. A mark-recapture method for measuring effects of spatial separation of horses on tabanid (Diptera) movement between hosts. J. Med. Entomol. 20: 301–305.

Foil, L. D. 1989. Tabanids as vectors of disease agents. Parasitol. Today 5: 88–96.

Foil, L. D., and J. R. Gorham. 2000. Mechanical transmission of disease agents by arthropods, pp. 461–514. *In* B. F. Eldridge and J. D. Edman (eds.), Medical entomology: a textbook on public health and veterinary problems caused by arthropods. Kluwer Academic Publishers, Dordrecht, Germany.

Foil, L. D., W. V. Adams, J. M. McManus, and C. J. Issel. 1987. Bloodmeal residues on mouthparts of *Tabanus fuscicostatus* (Diptera: Tabanidae) and the potential for mechanical transmission of pathogens. J. Med. Entomol. 24: 613–616.

French, D. M., T. F. McElwain, T. C. McGuire, and G. H. Palmer. 1998. Expression of *Anaplasma marginale* major surface protein 2 variants during persistent cyclic rickettsemia. Infect. Immun. 66: 1200–1207.

Futse, J. E., M. W. Ueti, D. P. Knowles, Jr., and G. H. Palmer. 2003. Transmission of *Anaplasma marginale* by *Boophilus microplus:* retention of vector competence in the absence of vector-pathogen interaction. J. Clin. Microbiol. 41: 3829–3834.

Hawkins, J. A., J. N. Love, and R. J. Hidalgo. 1982. Mechanical transmission of anaplasmosis by tabanids (Diptera: Tabanidae). Am. J. Vet. Res. 43: 732–734.

Howell, D. E., C. E. Sanborn, L. E. Rozeboom, G. W. Stiles, and L. H. Moe. 1941. Transmission of anaplasmosis by horseflies. Oklahoma Agricultural and Mechanical College, Stillwater, OK.

Knaus, R. M., L. D. Foil, C. J. Issel, and D. J. Leprince. 1993. Insect blood meal studies using radiosodium 24Na and 22Na. J. Am. Mosq. Control Assoc. 9: 264–268.

Lohr, C. V., F. R. Rurangirwa, T. F. McElwain, D. Stiller, and G. H. Palmer. 2002. Specific expression of *Anaplasma marginale* major surface protein 2 salivary gland variants occurs in the midgut and is an early event during tick transmission. Infect. Immunol. 70: 114–120.

Lotze, J. C. 1944. Carrier cattle as a source of infective material for horsefly transmission of anaplasmosis. Am. J. Vet. Res. 5: 164–165.

Morley, R. S., and M. E. Hugh-Jones. 1989. The effect of management and ecological factors on the epidemiology of anaplasmosis in the Red River Plains and south-east areas of Louisiana. Vet. Res. Commun. 13: 359–369.

Potgieter, F. T., B. Sutherland, and H. C. Biggs. 1981. Attempts to transmit *Anaplasma marginale* with *Hippobosca rufipes* and *Stomoxys calcitrans.* Onderstepoort. J. Vet. Res. 48: 119–122.

Ristic, M., and A. M. Watrach. 1963. Anaplasmosis. VI. Studies and a hypothesis concerning the cycle of development of the causative agent. Am. J. Vet. Res. 24: 267–277.

Scoles, G. A., M. W. Ueti, and G. H. Palmer. 2005a. Variation among geographically separated populations of *Dermacentor andersoni* (Acari: Ixodidae) in midgut susceptibility to *Anaplasma marginale* (Rickettsiales: Anaplasmataceae). J. Med. Entomol. 42: 153–162.

Scoles, G. A., A. B. Broce, T. J. Lysyk, and G. H. Palmer. 2005b. Relative efficiency of biological transmission of *Anaplasma marginale* (Rickettsiales: Anaplasmataceae) by *Dermacentor andersoni* (Acari: Ixodidae) compared to mechanical transmission by *Stomoxys calcitrans* (Diptera: Muscidae). J. Med. Entomol. 42: 668–675.

Wilson, B. H. 1968. Observations on horse fly abundance and the incidence of anaplasmosis in a herd of dairy cattle in southern Louisiana. Proceedings of the Fifth National Anaplasmosis Conference, 28–29 February 1968, Stillwater, OK.

Received 5 April 2007; accepted 18 August 2007.